EXHIBIT A

PURPOSE

To test the efficacy of aerosol IL-16 peptides, i.e., the 8-mer (RRKSLQSK) and the 16-mer (RRKSLQSKETTAAGDS), in the sheep model of allergic bronchoconstriction and airway hyperresponsiveness.

METHODS

All procedures were approved by the Mount Sinai Medical Center Animal Research Committee, which is responsible for assuring the humane care and use of experimental animals. The sheep used for this study had previously been shown to develop early and late airway responses and airway hyperresponsiveness to inhaled carbachol following inhalation challenge with Ascaris suum antigen.

Measurement of Airway Mechanics: The unsedated sheep were restrained in a cart in the prone position with their heads immobilized. After topical anesthesia of the nasal passages with 2% lidocaine solution, a balloon catheter was advanced through one nostril into the lower esophagus. The animals were intubated with a cuffed endotracheal through the other nostril using a flexible fiberoptic bronchoscope as a guide. (The cuff of the endotracheal tube was inflated only for the measurement of airway mechanics and during the aerosol challenges to prevent undue discomfort. This procedure has no effect on airway mechanics). Pleural pressure was estimated with the esophageal balloon catheter (filled with one ml of air) which was positioned 5-10 cm from the gastroesophageal junction. In this position, the end expiratory pleural pressure ranged between –2 and –5 cm H_20. Once the balloon was placed, it was secured so that it remained in position for the duration of the experiment. Lateral pressure in the trachea was measured with a sidehole catheter (inner dimension, 2.5 mm) advanced through and

positioned distal to the tip of the endotracheal tube. Transpulmonary pressure, the difference between tracheal and pleural pressure, was measured with a differential pressure transducer catheter system. For the measurement of pulmonary resistance (R_L), the proximal end of the endotracheal tube will be connected to a pneumotachograph (Fleisch, Dyna Sciences, Blue Bell, PA). The signals of flow and transpulmonary pressure will be recorded on an oscilloscope recorder which is linked to a computer for on-line calculation of R_L from transpulmonary pressure, respiratory volume (obtained by digital integration) and flow. Analysis of 5-10 breaths will be used for the determination of R_L. Immediately after the measurement of R_L, thoracic gas volume (V_{tg}) will be measured in a constant volume body plethysmograph to obtain specific lung resistance ($SR_L = R_L . V_{tg}$) in L x cm H_2O/LPS.

Aerosol Delivery Systems: Aerosols of Ascaris suum extract (diluted 20:1 with phosphate buffered saline; 82,000 PNU/ml) are generated using a disposable medical nebulizer (Raindrop^R, Puritan Bennett), which produces an aerosol with a mass median aerodynamic diameter of 3.2 μm (geometric standard deviation, 1.9) as determined by a 7 stage Andersen cascade impactor. The output from the nebulizer is directed into a plastic t-piece, one end of which is connected to the inspiratory port of a Harvard respirator. To better control aerosol delivery, a dosimeter consisting of a solenoid valve and a source of compressed air (20 psi) is activated at the beginning of the inspiratory cycle of the Harvard respirator system for 1 s. The aerosol is delivered at a tidal volume of 500 ml and a rate of 20 breaths per minute for 20 minutes. Each sheep will be challenged with an equivalent dose of antigen (400 breaths) in the control and drug trial. Carbachol aerosols are also generated with the nebulizer system described above.

For the carbachol dose response curves, measurements of SR_L will be repeated immediately after inhalation of buffer and after each administration of 10 breaths of increasing concentrations of carbachol solution (0.25%, 0.5%, 1.0%, 2.0% and 4.0% wt/vol). To assess airway responsiveness, the cumulative carbachol dose in breath units (BU) that increases SR_L 400% over the post-buffer value (i.e. PC_{400}) is calculated from the dose response curve. One breath unit is defined as one breath of a 1% w/v carbachol solution.

EXPERIMENTAL PROTOCOL

The same basic protocol was used for both studies. This basic protocol consisted of first obtaining baseline dose response curves to aerosol carbachol 1-3 days prior to antigen challenge. Then, on the day of antigen challenge, values of specific lung resistance (SR_L) were measured at baseline and, then, 30 min after drug or vehicle (0.9% saline) treatment. The animals were, then, challenged with Ascaris suum antigen and SR_L was remeasured immediately after challenge, hourly from 1-6 h after challenge and on the half-hour from 6 ½-8 h after challenge. Measurements of SR_L were obtained 24 h after challenge followed by the 24h post- challenge dose response curve.

Studies differed in time of treatment and treatment dose. In the 16-mer study, animals were treated with 3mg of the 16-mer (dissolved in 5 ml 0.9% saline) by aerosol 30 min before antigen challenge. In the 8-mer study, animals were treated with 3mg of the 8-mer (dissolved in 5 ml 0.9% saline) by aerosol, once a day for 3 days and, then, again on the 4th (antigen challenge day) 30 min before antigen challenge.

To assess airway responsiveness, the cumulative carbachol dose that increases SR_L 400% over the post-buffer value (i.e. PC_{400}) is calculated.

RESULTS

3 mg 16-mer aerosol once at 30 min.

For the 16-mer, the top figure illustrates the time course of the antigen-induced responses and the bottom figure the effects on airway responsiveness in the two sheep treated once at 30 min prior to antigen challenge. For the 16-mer (top figure) aerosol treatment of 3mg protected against the antigen-induced LAR. Consistent with the protection against the late response was the protection against the antigen-induced AHR (bottom figure).

3 mg 8-mer once a day for 4 days.

For the 8-mer, the top figure illustrates the time course of the antigen-induced responses and the bottom figure the effects on airway responsiveness in the two sheep treated once a day for four days with 8-mer aerosol. There was no effect on the EAR, however, the 8-mer blocked the LAR to allergen in these animals (top figure). Consistent with the protection against the late response was the protection against the antigen-induced AHR (bottom figure).

FIGURE LEGEND

16-mer Top Figure.

Time course of antigen-induced changes in specific lung resistance (SR_L) in two sheep treated with 3mg 16-mer aerosol once 30 min prior to antigen challenge. Responses are compared to the animals' historical control (Control)

16-mer Bottom Figure:

Effect of 16-mer on airway responsiveness. A decrease in the PC400 from baseline indicates the development of airway hyperresponsiveness (control)

whereas no change from baseline indicates that airway hyperresponsiveness does not develop.

8-mer Top Figure.

Time course of antigen-induced changes in specific lung resistance (SR_L) in two sheep treated with 3mg 8-mer aerosol once daily for 3 days and, then, again on the 4th (antigen challenge day) 30 min before antigen challenge. Responses are compared to the animals' historical control (Control).

8-mer Bottom Figure:

Effect of 8-mer on airway responsiveness. A decrease in the PC400 from baseline indicates the development of airway hyperresponsiveness (control) whereas no change from baseline indicates that airway hyperresponsiveness does not develop.